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TOTAL CONCENTRATION OF FREE AMINO ACIDS AND THE CORRESPONDING MORPHOGENETIC CHANGES DURING THE EMBRYOGENESIS OF *CHILOMENESE SEXMACULATA* FABR. (COLEOPTERA: COCCINELLIDAE)

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Abstract.—The concentration of free amino acid (FAA) pool in relation to the various morphogenetic events occurring during embryogenesis of *Chilomenes sexmaculata* Fabr. was determined at different incubation periods. During the first 12 hours of embryogenesis, when the zygote undergoes an active cleavage, the concentration declines gradually. However, it rises sharply at 15 hours of development when the cleavage energids undergo an active migratory activity. The amount of FAA decreases at 24 hours and remains almost steady up to 35 hours of incubation when the embryo starts elongation and segmentation. From then on there is a continuous increase in FAA up to 75 hours followed by a declining trend towards hatching as the yolk gets depleted.

Introduction

Extensive reviews on the biochemical investigations pertaining to the nitrogen metabolism during embryogenesis of insects have earlier been made by Chen (1962, 1966) and Corrigan (1970). To get a greater insight into the development of an embryo it is necessary to elucidate the relationship of biochemical changes to the corresponding morphogenetic events occurring during this period. Since the major processes underlying differentiation at the embryonic level aim at protein synthesis which in turn depends on the supply of free amino acids (FAA) from the yolk reserves, FAA thus have a distinct relationship to morphogenesis.

The present attempt unveils a relationship between the total concentration of FAA and the corresponding morphogenetic events occurring during the different incubation periods in *Chilomenes sexmaculata* Fabr.

Materials and Methods

The adults of *C. sexmaculata* were collected from the *Calotrips procera* plants during the months of November to February around Patiala (India) and were reared in the laboratory according to Sarswat (1976) on *Aphis neri*. Numerous batches of eggs were obtained, incubated at 28 ± 2°C for

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Table 1. Concentration of FAA pool and various morphogenetic events occurring during embryogenesis of *Chilomenes sexmaculata* Fabr.

Incubation period (hr)	μM FAA/100 eggs (±SD)*	Morphogenetic event
0.25	1.51 ± 0.030	Maturation of the male as well as female pronucleus (Fig. 1).
1.00	1.48 ± 0.029	Fusion of male and female nucleus (Fig. 2).
3.00	1.34 ± 0.027	Cleavage of the zygote, giving rise to 4-energids stage (Fig. 3).
5.00	1.46 ± 0.029	8-cleavage energids stage (Fig. 4).
7.50	1.16 ± 0.023	The process of cleavage continues.
12.00	1.11 ± 0.022	240 ± 16 energids stage (Fig. 5).
15.00	1.77 ± 0.035	Migration of the cleavage energids towards the periplasm starts (Fig. 6).
19.00	1.68 ± 0.035	A syncytial primary epithelium is formed (Fig. 7).
24.00	1.37 ± 0.028	A regular layer of primary epithelial cells observed (Fig. 8).
31.00	1.39 ± 0.028	The ventro-posterior portion of the primary epithelium becomes thickened, the cells being columnar, so as to give rise to the definitive germ primordium (ventral plate) (Fig. 9).
35.00	1.32 ± 0.026	Elongation of the germ band or ventral plate takes place and the germ band becoming faintly distinguishable into an anterior protocephalon and the posterior protocorm (Fig. 10).
45.00	1.42 ± 0.028	Further elongation of the germ band resulting in the formation of three protocormic regions (Fig. 11).
60.00	1.45 ± 0.029	The germ band becomes segmented with delineated appendages (Fig. 12).
72.00	1.48 ± 0.029	
75.00	1.55 ± 0.030	An active growth period, the appendages further develop and the process of cephalization starts, secondary yolk cleavage, yolk poly-hedra formed.
90.00	1.35 ± 0.026	The appendages become fully formed and the head also becomes distinct with the usual appendages (Fig. 13).
100.00	1.30 ± 0.026	Fully formed embryo.
110.00	1.22 ± 0.024	Hatching takes place.

* Standard deviation. Each reading is an average of three determinations.

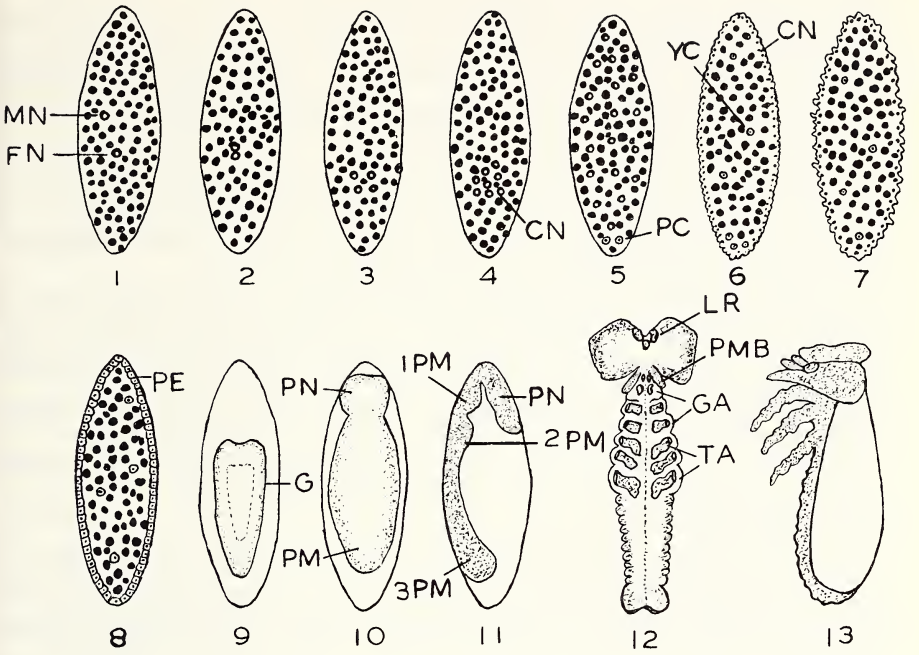

Fig. 1. Abbreviations: CN, cleavage energid; FN, female pronucleus; G, germ band; GA, gnathal appendages; LR, labral appendages; MN, male pronucleus; PC, pole cell; PE, primary epithelium; PM, protocorm; 1PM, 2PM, 3PM, 1st, 2nd and 3rd protocormic regions; PMB, premandibular appendages; PN, protocephalon; TA, thoracic appendages; YC, yolk cell.

definite periods and subsequently fixed in Carl's fixative at 60°C for 2 hours. The permanent preparations were made according to the method of Rempel and Church (1971) to observe various developmental changes in the embryo.

The FAA extracts from the various egg samples at specific incubation periods were prepared according to Sidhu and Kang (1979). The quantitation of FAA pool was made by the method of Troll and Cannon (1953).

Results and Discussion

The concentration of FAA pool and the various morphogenetic events occurring during the embryogenesis of *Chilomenes sexmaculata* Fabr. are recorded in Table 1.

At 0 hour, i.e., immediately after oviposition, the concentration was 1.51 ± 0.030 μmoles/100 eggs, which decreased three hours later to 1.34 ± 0.027 μmoles/100 eggs. This decrease in FAA concentration corresponds to the period when the exochorion of the newly laid egg becomes hard and

dark in color, apparently indicating the utilization of FAA in this process. It has further been noted that there is a continuous decrease in FAA concentration up to 12 hours of incubation, i.e., during the active cleavage activity when the zygote nucleus undergoes multiple synchronous divisions. This decrease suggests that the breakdown of yolk proteins has not yet started and the need of amino acids for protein synthesis is met from the already existing FAA pool accumulated during maturation of egg. The initiation of protein synthesis immediately after fertilization has earlier been reported by Lockshin (1966) in some coleopterous insects.

At about 15 hours of incubation, when energids start migrating, the concentration of FAA suddenly increases to a fairly high value (1.77 ± 0.035 μmoles/100 egg). This increase is due perhaps to the lysis of yolk proteins, with the result that the mixing-up of the yolk contents facilitates the peripheral migration of the cleavage energids. This type of increase in FAA concentration has been observed by Chen and Briegel (1965) in *Culex pipens morestus* at a stage when blastoderm formation and elongation of germ band (Idris 1960) takes place.

The FAA concentration sharply decreases (1.37 ± 0.28 μmoles/100 eggs) at 24 hours of incubation when a regular primary epithelial layer gets established, revealing a higher rate of protein synthesis in comparison with the breakdown of yolk proteins. Thereafter the concentration remains almost steady up to 34 hours of development. During this period the differentiation of the ventral plate or the germ band is completed.

At 45 hours of development the amount of FAA again rises (1.42 ± 0.028 μmoles/100 eggs), when the germ band undergoes further elongation and primary segmentation. This trend in FAA concentration increase was observed in the following 30 hours of development. The peak is reached at 75 hours of incubation when the embryo shows an active development. This increase in concentration can be attributed to an active lysis of the yolk proteins, corresponding to an active growth period when cephalization as well as the phenomenon of secondary yolk cleavage takes place.

After this period, the FAA concentration shows a continuous decline when the appendages become fully formed and the head becomes quite distinct. This decline in concentration prior to hatching is due perhaps to the fact that the yolk reserves become increasingly exhausted at this stage and the supply of amino acids can no longer keep pace with the demand for more amino acids for protein synthesis. Chen (1966) and Indira (1963) have also observed a similar type of decrease in FAA concentration towards hatching.

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